Impact of the renin–angiotensin system on cerebral perfusion following subarachnoid haemorrhage in the rat

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1. This study investigated the effects of blocking the AT_1 angiotensin receptors with irbesartan, either peripherally or centrally, on systemic blood pressure, intracranial pressure and cerebral perfusion pressure following experimental subarachnoid haemorrhage (SAH) in urethane-anaesthetized rats. Sympathetic nervous activation was determined by measuring plasma noradrenaline levels.

2. In untreated animals, SAH induced a sustained increased in intracranial pressure from 2.1 ± 0.3 to 16 ± 2 mmHg (3 h, $P < 0.001$). Cerebral perfusion pressure was reduced by 20% ($P < 0.001$), this reduction being maintained for 3 h. Sympathetic activation was evident in the high level of plasma noradrenaline measured 3 h post-SAH (751 ± 104 *vs.* 405 ± 33 pg ml^{-1}, $P < 0.05$).

3. Acute peripheral pretreatment with irbesartan (3 mg kg^{-1}, I.V.) prevented the rise in plasma noradrenaline and further aggravated the decrease in cerebral perfusion pressure by producing transient systemic hypotension (blood pressure was 85 ± 6 mmHg at 2 h post-SAH *vs.* 100 ± 3 mmHg, $P < 0.01$).

4. Intracisternal pretreatment with irbesartan (0.035 mg) did not prevent the rise in plasma noradrenaline post-SAH but enhanced the rise in intracranial pressure by 75% compared with untreated animals.

5. This study demonstrates that peripheral endogenous angiotensin II interacts with the sympathetic nervous system in order to maintain an adequate cerebral perfusion following SAH. Endogenous angiotensin II in the brain seems to exert a protective effect by counteracting the elevation in intracranial pressure that occurs following experimental SAH.

Subarachnoid haemorrhage (SAH) is a serious condition characterized by a high level of morbidity and mortality. Indeed, some studies have documented the mortality to be as high as 45% in the 30 days following the trauma (Broderick *et al.* 1994). Despite improved microsurgical techniques this high level of mortality remains, largely as a consequence of rebleeding, but also due to the development of cerebral vasospasm and/or hydrocephalus resulting in delayed cerebral ischaemia. Indeed, it has been reported that cerebral ischaemia arises as a result of a combination of cerebral vasospasm and raised intracranial pressure, leading to a reduction in cerebral perfusion (Brinker *et al.* 1992).

The observation that patients presenting elevated plasma renin levels experience a higher incidence of mortality and morbidity than those with lower plasma renin (Neil-Dwyer *et al.* 1980) indicates that the renin–angiotensin system may be involved in some of the deleterious consequences of SAH. Whether this occurs as a result of angiotensin II generating cerebral vasospasm remains to be seen. Interestingly, in experimental studies of SAH in rats (Honda *et al.* 1997) and dogs (Andrews *et al.* 1982), delayed cerebral vasospasm is reversed by the use of an angiotensin-converting enzyme inhibitor.

The role of the central angiotensin II system in the pathology of SAH is not well known. The combined activation of both the peripheral and central angiotensin systems may play a role in the pathophysiology following SAH. Indeed, increases in both angiotensin II-like immunoreactivity and angiotensin II receptor content at the circumventricular organs, organum vasculosum lamina terminalis and subfornical organs in the rat following SAH have been demonstrated (Acikgoz *et al.* 1996). It is therefore feasible that antagonists of angiotensin II receptors may be of benefit in the management of patients following SAH. Contrary to this view, in some recent experiments we found that activation of the renin–angiotensin system following

experimental SAH appeared to be beneficial, as systemic pretreatment with losartan resulted in substantially increased mortality (Fassot *et al.* 1999). The mechanism by which angiotensin II exerts its beneficial role remains to be determined. In the present study, using an acute model of SAH in rats, we investigated the relative importance of peripheral and central angiotensin II in maintaining intracranial pressure and cerebral perfusion, and their interaction with the sympathetic nervous system.

METHODS

Experiments were performed on male Sprague-Dawley rats (380–420 g) bred and housed at the Baker Medical Research Institute. All procedures were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific purposes. All rats were housed under controlled temperature and humidity and a 12:12 h dark–light cycle with free access to food and water.

Surgical method

Anaesthesia was induced with a mixture of sodium pentobarbitone (30 mg kg^{-1}, I.P.) and brietal (30 mg kg^{-1}, I.P.) and subsequently maintained with urethane (1 g kg^{-1}, infused at a rate of 1.2 ml h^{-1}) for about 10 min. When required, anaesthesia was supplemented with urethane. Body temperature was maintained at 37 °C with a heating pad.

A catheter was placed in the right femoral artery to measure systemic arterial pressure and to obtain blood for noradrenaline analysis. The right femoral vein was cannulated for infusion of either anaesthetic or drugs. Animals were then placed in a stereotaxic frame and were kept with the head in a nose-down position. The atlanto-occipital membrane was exposed. Two hypodermic needles (25G and 30G1/2) connected to a catheter were mounted on the manipulating arm of the stereotaxic device. The 25G needle was inserted rostrally and was used to measure intracranial pressure. The 30G1/2 was inserted 0.15 mm anterior to the other needle and was used to inject blood and drugs. Both needles were advanced into the cisterna magna until a satisfactory intracranial pressure waveform was obtained. Systemic blood pressure and intracranial pressure were both measured using a TranStar transducer (Medex, Hilliard, OH, USA) connected to a PowerLab recording unit (Neomedix Systems, Warriewood, NSW, Australia). Cerebral perfusion pressure was calculated as the difference between mean blood pressure (MBP) and intracranial pressure.

Experimental protocol

Blood pressure and intracranial pressure were constantly recorded for the duration of the experiments. Baseline recordings were obtained for 15 min prior to induction of SAH and subsequently for a 3 h period post-SAH. Subarachnoid haemorrhage was induced by injection of freshly drawn homologous, non-heparinized arterial blood (0.3 ml) into the cisterna magna over approximately 1 min. Sham-treated animals received 0.3 ml of saline instead of blood.

Animals were divided into four experimental groups. In group 1 ($n = 7$), the effects of subarachnoid injection of saline were examined. In group 2 ($n = 16$), animals were subjected to subarachnoid injection of 300 μl blood and did not receive any pharmacological intervention. In the third group ($n = 7$), the effect of peripherally administered irbesartan (3 mg kg^{-1}, I.V.), initiated 15 min prior to intracisternal (I.C.) injection of blood (via the cisterna magna) was investigated. Animals received a second dose of irbesartan 1.5 h after the initial dose to ensure the integrity of the angiotensin II receptor blockade throughout the experiment. In the fourth experimental group ($n = 14$), the effect of I.C. injection of irbesartan (0.035 mg (5 μl)$^{-1}$), initiated 15 min prior to induction of SAH, was investigated. Animals received the same dose of irbesartan 1.5 h after the initial dose. Injection via this site results in the preferential entry of drug along the ventral side of the brain, especially into the hypothalamus and brainstem (Proescholdt *et al.* 2000). Three hours after SAH, an arterial blood sample (1 ml) was taken for subsequent plasma noradrenaline analysis.

In addition, an extra group of rats ($n = 8$) was used to investigate the effects of I.C. administration of irbesartan on intracranial pressure, blood pressure and plasma noradrenaline. Animals underwent the same surgical procedure as described above. After baseline measurements of blood pressure and intracranial pressure, irbesartan (0.035 mg (5 μl)$^{-1}$) was injected into the cisterna magna. A second identical dose of irbesartan was given 1 h after the first injection. Blood pressure and intracranial pressure were measured over a 3 h period and a 1 ml blood sample was taken from the femoral artery for subsequent plasma noradrenaline analysis.

At the end of the expeiriments, the rats were killed with an overdose of pentobarbitone.

Noradrenaline was extracted from plasma with alumina adsorption, separated by high-performance liquid chromatography and the amount quantified by electrochemical detection according to previously described methods (Eisenhofer *et al.* 1986). The chromatographic system consisted of a Model 480 High Precision Pump, a Model Gina autosampler, a Model STH 585 column oven, a Chromeleon 3.03 Chromatography Data System (Gynkotek, Germering, Germany), a Model 5100A coulometric detector equipped with a Model 5021 conditioning cell and a Model 5011 analytical cell (Environmental Sciences Associates, Chelmsford, MA, USA) and a 25 cm Altex Ultrasphere column (ODS 4.6 mm × 25 cm, 5 μm particle size, Beckman Instruments Inc., Fullerton, CA, USA). Analysis was performed at 24 °C with the operating potentials set at +0.35 V for the guard cell and −0.35 and +0.29 V for detectors 1 and 2, respectively. All measurements were made using the oxidizing potential applied at detector 2 and compounds in plasma were identified by their retention behaviour compared with that of authentic standard solutions.

Statistics

Values were expressed as means ± S.E.M. Because the experimental design involved multiple measurements in different groups of animals, a split plot (nested) repeated measure analysis of variance was used for haemodynamic parameters. The total sums of squares (SS) were divided into between-groups SS and within-groups SS. Comparisons within each group were made by using orthogonal contrasts. The F ratio of each contrast was calculated as the mean square (MS) for the contrast divided by the total residual MS of the four groups. In this way, the estimate of the within-group variance was made with a contribution from all the groups rather than the individual. Comparisons within each group were made by using orthogonal contrasts. Between-group comparisons were made using the F ratio of the between-group MS divided by the rows × groups interaction. The effect of the different treatments was determined from the group × treatment interaction. A comparison of the different control periods was also made between groups. For the noradrenaline measurements, a one-way analysis of variance was used.

RESULTS

Effects of SAH or saline injection into the subarachnoid space in untreated animals

Experimental SAH, induced by the injection of 0.3 ml of blood into the subarachnoid space, resulted in a transient, but pronounced, increase in intracranial pressure (142 ± 16 mmHg) within ~70 s of the blood injection. This was associated with a transient rise in MBP of 46 ± 7 mmHg and in HR of 41 ± 8 beats min^{-1} (bpm). Cerebral perfusion pressure was reduced following SAH (Fig. 1). Intracranial pressure remained elevated for the entire experimental period (at 3 h, 16 ± 2 *vs.* 2.1 ± 0.3 mmHg, $P < 0.001$, Fig. 2). Following the initial rise in blood pressure, MBP was subsequently reduced and remained so for the hour following SAH (93 ± 2 *vs.* 101 ± 2 mmHg, $P = 0.001$). Blood pressure had returned to baseline 2 h following SAH. For the entire experimental period cerebral perfusion pressure remained reduced (at 3 h, 85 ± 4 *vs.* 101 ± 2 mmHg, $P < 0.001$, Fig. 2). Heart rate returned to baseline levels within 5 min of SAH and remained stable throughout the experiment (Fig. 2). Four of the 16 animals died following SAH, three within 10 min and one 40 min post-SAH. Sham-treated animals received 0.3 ml of saline into the subarachnoid space. Within ~40 s, the saline injection induced a rise in intracranial pressure of 54 ± 5 mmHg. Intracranial pressure then formed a plateau that remained at this level for approximately 20 s. This was associated with a transient reduction in MBP (−10 ± 3 mmHg) and heart rate (−10 ± 9 beats min^{-1} (bpm), Fig. 1). Following subarachnoid saline injection, intracranial pressure returned to normal within 5 min. No further changes in intracranial pressure, MBP or HR were observed for the remainder of the experimental period (Fig. 2).

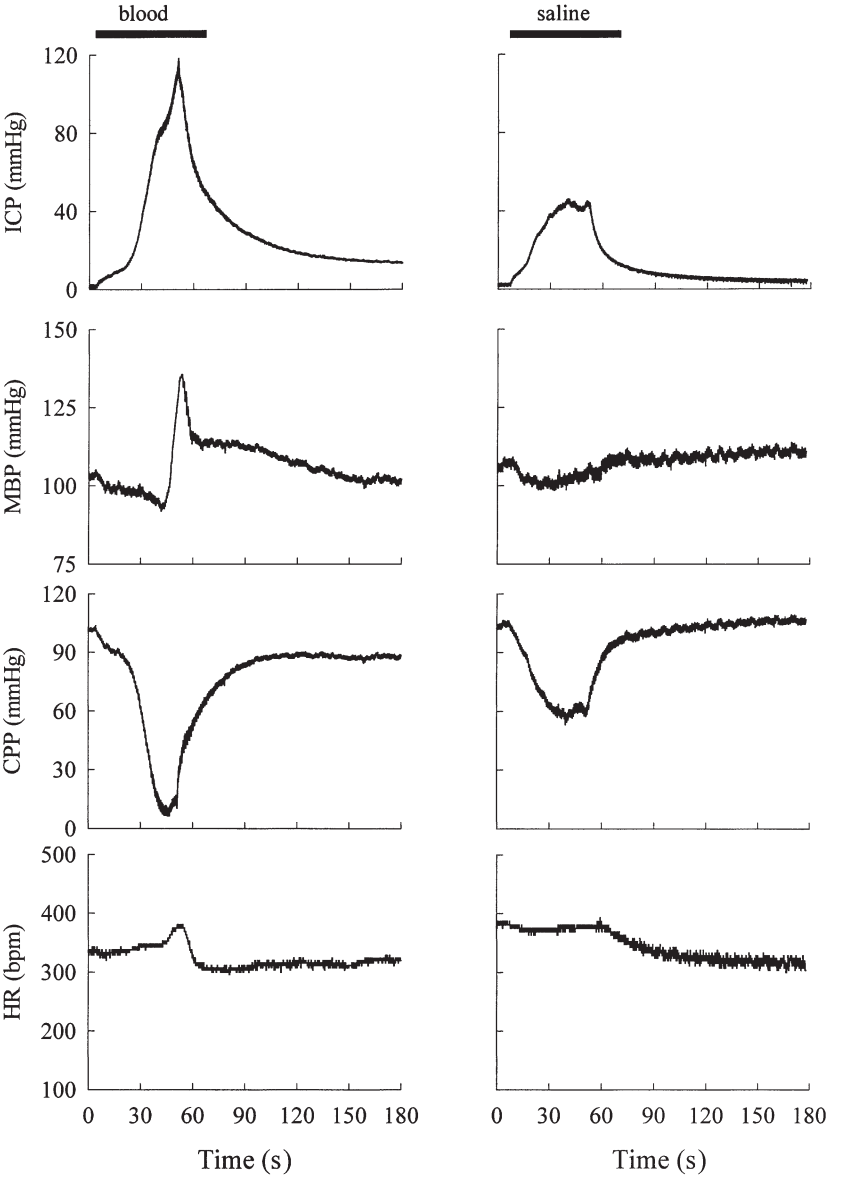

Figure 1. Effects of injection of blood (subarachnoid haemorrhage, SAH) or saline on untreated animals

Examples of intracranial pressure (ICP), mean blood pressure (MBP), cerebral perfusion pressure (CPP) and heart rate (HR) changes after injection of blood or saline into the subarachnoid space. Left panel, blood; right panel, saline.

Indicative of a pronounced sympathoexcitation, plasma levels of noradrenaline were markedly increased 3 h following the subarachnoid injection of blood (751 ± 104 *vs.* 405 ± 33 pg ml^{-1}, $P < 0.05$ compared with sham-treated animals, Fig. 3).

Effects of SAH after peripheral AT_1 receptor blockade with irbesartan

Irbesartan (3 mg kg^{-1}, I.V.) administration 15 min prior to induction of SAH did not alter the basal levels of MBP, HR or intracranial pressure. The subarachnoid injection of blood resulted in an acute rise in intracranial pressure, MBP and HR similar to that observed in untreated SAH animals. Intracranial pressure came back to 14 ± 2 mmHg at 30 min and remained elevated for the 3 h experimental period (15 ± 1 mmHg at 3 h *vs.* 2.8 ± 1.0 mmHg, $P < 0.001$ Fig. 2). The time course of intracranial pressure fluctuation following SAH in I.V. irbesartan-treated animals was similar to that observed in untreated animals ($F_{1,140} = 0.10$, $P = 0.75$). Blood pressure was reduced following subarachnoid blood injection (85 ± 5 mmHg at 30 min *vs.* 100 ± 3 mmHg, $P < 0.01$). This fall in blood pressure was evident for 2 h following SAH. There was a significant difference between the time course of blood pressure after SAH between untreated and I.V. irbesartan-treated groups ($F_{1,140} = 98.36$, $P = 0.004$). Cerebral perfusion pressure was reduced 30 min following SAH (72 ± 5 mmHg *vs.* 98 ± 4 mmHg, $P < 0.001$) and remained low for the 3 h recording period. The reduction in cerebral perfusion pressure throughout the 3 h recording session in I.V. irbesartan-treated rats was more pronounced than that observed in untreated SAH rats ($F_{1,140} = 7{,}9$, $P = 0.006$). Heart rate returned to the baseline level within 5 min and was not altered subsequently (Fig. 2).

Three hours following SAH in I.V. irbesartan-treated rats, plasma levels of noradrenaline were not different to those observed in sham-treated animals (Fig. 3).

The effectiveness of peripheral angiotensin II receptor blockade with irbesartan was confirmed at the end of the experiment by the lack of response of blood pressure to I.V. administration of 100 ng of angiotensin II (data not shown).

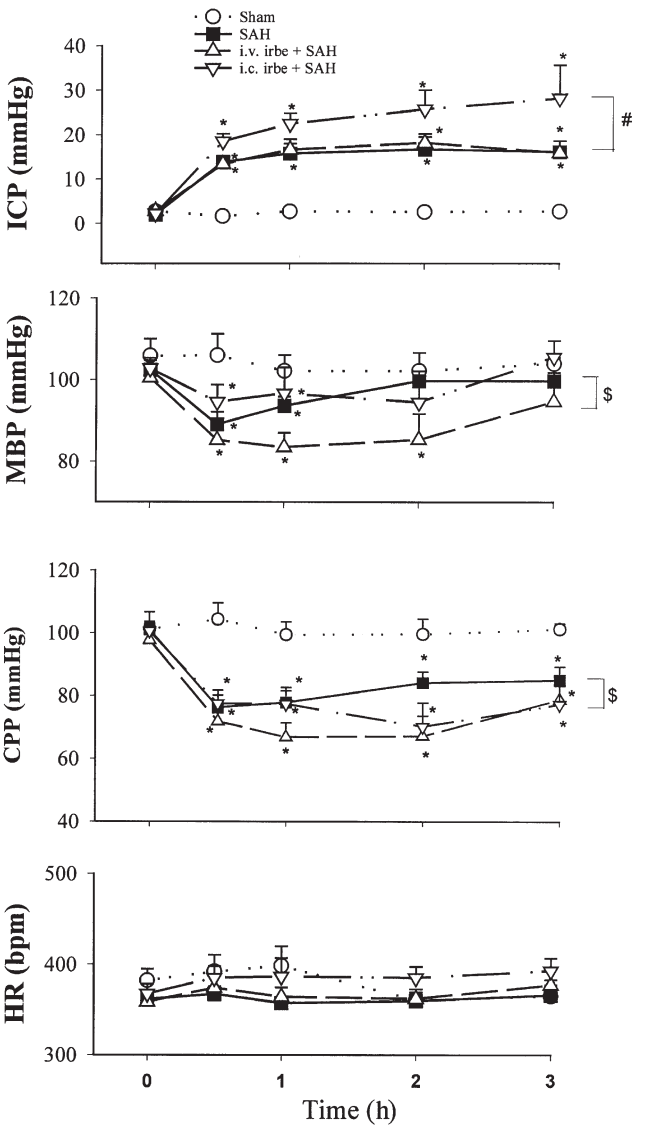

Figure 2. Effects of injection of blood (SAH) or saline on intracranial, blood and cerebral perfusion pressures

Intracranial pressure (ICP), mean blood pressure (MBP) and cerebral perfusion pressure (CPP) changes after the injection of 0.3 ml of blood (SAH) or saline (Sham) into the subarachnoid space. *$P < 0.05$ *vs.* control. #$P < 0.001$ group SAH + I.C. irbesartan *vs.* SAH. $$P < 0.01$ group SAH + I.V. irbesartan (irbe) *vs.* SAH.

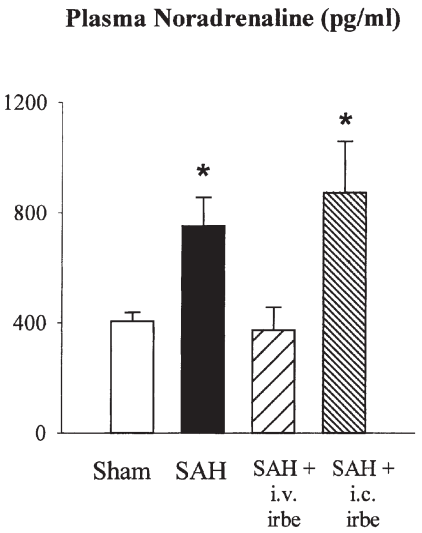

Figure 3. Effects of injection of blood (SAH) or saline on plasma noradrenaline

Plasma levels of noradrenaline 3 h after the injection of 0.3 ml of saline (Sham) or blood (SAH) into the subarachnoid space. Irbe, irbesartan. *$P < 0.05$ *vs.* sham treatment.

Effects of SAH after central AT_1 receptor blockade with irbesartan

Injection of irbesartan (0.035 mg) into the cisterna magna 15 min prior to SAH did not alter the basal levels of MBP, HR or intracranial pressure. Experimental SAH resulted in an acute rise in intracranial pressure, MBP and HR in these animals that was similar in magnitude to that observed following SAH in untreated animals. Intracranial pressure was elevated 30 min following SAH (19 ± 2 *vs.* 2.76 ± 0.59 mmHg, $P < 0.001$), and subsequently increased further to 28 ± 9 mmHg 3 h after the subarachnoid injection of blood. The increase in intracranial pressure over the 3 h experimental period was more pronounced than that observed in untreated SAH animals ($F_{1,140} = 19$, $P < 0.001$). Mean blood pressure was significantly lower during the first hour following SAH (91 ± 6 *vs.* 104 ± 4 mmHg, $P < 0.01$) but returned to a normal level following this time point. The time course of MBP over the 3 h recording period was not different from the MBP profile exhibited by the untreated SAH animals ($F_{1,140} = 0.003$, $P = 0.95$). Cerebral perfusion pressure was significantly diminished throughout the experimental period (75 ± 4 mmHg at 30 min *vs.* 102 ± 4 mmHg, $P < 0.001$) and was similar to that observed in untreated SAH rats ($F_{1,40} = 3.6$, $P = 0.061$). Heart rate returned to normal within 5 min following SAH and remained unchanged for the remainder of the experiment (data not shown). Six of the 14 animals died during the recording period, four within the first 10 min post-SAH and two after 2.5 h.

Three hours following experimental SAH the plasma level of noradrenaline was higher than that observed in sham-treated animals (Fig. 3).

Effect of I.C. administration of irbesartan on intracranial pressure, MBP, HR, plasma noradrenaline level and angiotensin II response

The effects of I.C. injection of irbesartan on intracranial pressure, MBP, HR and plasma noradrenaline were investigated in eight animals that were not submitted to any injection of saline or blood into the subarachnoid space. Baseline levels of intracranial pressure, MBP and HR were 2.27 ± 0.38 mmHg, 103 ± 4 mmHg and 390 ± 10 beats min^{-1} respectively. The injection of irbesartan (0.035 mg, performed after baseline recording and 1.5 h later) did not influence any of these parameters during the 3 h recording period. Plasma levels of noradrenaline in these animals were 256 ± 101 pg ml^{-1}.

Prior to I.C. injection of irbesartan, I.C. administration of 100 ng of angiotensin II induced a rise in blood pressure of 22 ± 2 mmHg. One hour after the I.C. administration of irbesartan, the same dose of angiotensin II induced a markedly diminished rise in blood pressure of 4 ± 2 mmHg (paired *t* test, $P < 0.01$). On average, the I.C. injection of 5 μl of irbesartan followed by injection of 10 μl of saline induced a rise in intracranial pressure of approximately 3 mmHg. This rise in intracranial pressure was normalized within 3 min.

DISCUSSION

The demonstration that delayed cerebral vasospasm following experimental subarachnoid bleeding is reversed following angiotensin-converting enzyme inhibition (Andrews *et al.* 1982; Honda *et al.* 1997), combined with the observation that plasma renin levels are predictive of mortality and morbidity (Neil-Dwyer *et al.* 1980), indicates that the renin–angiotensin system may be involved in some of the deleterious consequences of SAH. Perhaps contrary to this view, but in agreement with our previous observations (Fassot *et al.* 1999), the present study indicates that both peripheral and central angiotensin II seem essential in order to maintain cerebral perfusion in the face of raised intracranial pressure. In addition, the sympathoexcitation that is evident following SAH (Lambert *et al.* 2000; Naredi *et al.* 2000) requires an intact peripheral renin–angiotensin system.

In the present study, experimental SAH resulted in a 7-fold increase in intracranial pressure with a concomitant 20 % reduction in cerebral perfusion pressure. This was maintained for at least 3 h and was associated with an activation of the sympathetic nervous system, as indicated by elevated plasma concentrations of noradrenaline. Such sympathoexcitation following SAH is consistent with some of our previous experimental (Lambert *et al.* 2000) and clinical (Naredi *et al.* 2000) investigations. In the present study we measured plasma noradrenaline levels 3 h after experimentally induced SAH. We have previously demonstrated in conscious animals that sympathoexcitation, as estimated from analysing mid-frequency oscillations in blood pressure, developed progressively following SAH, reaching a significant level 2–3 h subsequent to the trauma (Lambert *et al.* 2000, 2001). In fact the sympathoexcitation observed after 2 h is not significantly different to that observed 3 h after the trauma (Lambert *et al.* 2001). Using direct recording of the sympathetic nerves, Fedina *et al.* demonstrated that the sympathetic overactivity caused by SAH in cats was triggered by elevation in intracranial pressure (Fedina *et al.* 1986). This view seems contrary to our clinical experience where we found no relation between the level of intracranial pressure and the rate of noradrenaline spillover to plasma (Naredi *et al.* 2000). The initial rise in blood pressure induced by SAH is prevented by prior α-adrenergic blockade (van Wylen & D'Alecy, 1985) and is believed to be mediated via alterations in neuronal activity in the brainstem (Dampney & Moon, 1980; Gebber & Barman, 1985) in response to local ischaemia (Astrup *et al.* 1981), or more particularly, hypoxia (Sun & Reis, 1994). We found that injection of saline into the cisterna magna increased intracranial pressure until a pressure plateau was formed.

The infusion of blood resulted in a more pronounced elevation in intracranial pressure without the concomitant development of a pressure plateau. This observation is consistent with previous studies performed in cats (Brinker *et al.* 1992). The greater increase in intracranial pressure following blood injection occurs as a result of the acute impairment of cerebrospinal fluid absorption coupled with a reduction in the intracranial space buffering capacity (Brinker *et al.* 1990).

We aimed to differentiate between the peripheral and central effects of the AT_1 receptor antagonist irbesartan on the maintenance of cerebral perfusion pressure following SAH. Given that systemically administered irbesartan may access angiotensin II receptors in the brain (Culman *et al.* 1999), we used a small dose of irbesartan, sufficient to block the peripheral AT_1 receptors but with little effect on central AT_1 receptors (Culman *et al.* 1999). Not surprisingly, peripheral injection of irbesartan was without effect on systemic blood pressure. Indeed, AT_1 receptor antagonists are without any discernible effect on blood pressure in normotensive patients (Schmitt *et al.* 1998). We found that irbesartan administration resulted in a greater reduction in blood pressure following SAH compared with untreated animals subjected to SAH. An increase in plasma renin activity has been documented 3 h after experimental SAH (Fassot *et al.* 1999). In the present study the reduction in blood pressure within the first hour following SAH in untreated animals may promote the release of renin from the kidney via interaction with the sympathetic nervous system.

Following experimental SAH, animals pretreated with I.V. irbesartan did not exhibit any alteration in plasma noradrenaline levels, which remained similar to those observed in sham-treated animals. In addition, blood pressure recovery was retarded compared with that of untreated animals. Taken together these observations suggest the absence of any discernible sympathoexcitation occurring in response to SAH in this group of animals. Whether the lack of sympathoexcitation occurs as a result of irbesartan's ability to reduce or inhibit sympathetic nervous activation, as previously observed in pithed rats (Moreau *et al.* 1993), remains to be seen. Our observations suggest that the sympathoexcitation that occurs in response to experimental SAH is dependent, at least in part, on an intact renin–angiotensin system. Interaction between the sympathetic nervous and renin–angiotensin systems has previously been described (Reid, 1992). Indeed, there is evidence supporting an inhibitory influence of angiotensin AT_1 receptor blockade (Rongen *et al.* 1998) and converting enzyme inhibitors (Grassi *et al.* 1998) on the sympathetic nervous system.

Given that the area postrema lacks a blood–brain barrier it may be exposed to circulating angiotensin II and hence may interact with neurones in the medial nucleus tractus solitarius resulting in the modulation of autonomic control of the cardiovascular system (Bishop & Hay, 1993; Bishop *et al.* 1991). In addition, circulating angiotensin II may also act on the subfornical organ and organum vasculosum lamina terminalis in the forebrain. Likewise, peripheral administration of irbesartan may reach the AT_1 receptors of the area postrema and modify sympathetic nervous activity. Alternatively, peripheral irbesartan may act to prevent the induction of sympathoexcitation by either inhibiting the effect of angiotensin II to: (1) augment the postsynaptic response to noradrenaline (Peach, 1977) and/or (2) enhance the secretion of catecholamines from the adrenal medulla (Peach *et al.* 1969). In both the area postrema and nucleus tractus solitarius, brain regions implicated in the interaction between the autonomic nervous and brain angiotensin systems, the predominant angiotensin II receptor is the AT_1 subtype (Tsutsumi & Saavedra, 1991). The possibility of angiotensin II acting via AT_2 receptors does merit some attention since it has been reported that they also play a role in mediating catecholamine secretion at the level of the adrenal medulla (Martineau *et al.* 1999).

When directly injected into the cisterna magna, we found that irbesartan failed to prevent the sympathoexcitation induced by SAH. The impact of central angiotensin receptor blockade on sympathetic nervous activity remains conjectural. In response to central angiotensin receptor blockade, sympathetic nervous activity has been observed to remain unchanged (Dorward & Rudd, 1991), or to be increased (Gaudet *et al.* 1998). In the present study, I.C. injection of irbesartan did not influence resting blood pressure *per se* or increase arterial noradrenaline levels in animals not subjected to SAH. Furthermore, following SAH, systemic blood pressure was similar to that observed in untreated SAH animals. Our observation of an enhanced intracranial pressure rise following SAH after I.C. pretreatment with irbesartan is intriguing. The level of intracranial pressure was 75% higher than that observed in untreated SAH rats. Elevated intracranial pressure could result from a dilatation of cerebral arteries induced by AT_1 receptor blockade. As cerebral autoregulation is impaired after SAH (Rasmussen *et al.* 1992), dilatation of cerebral arteries without a decrease in systemic blood pressure may contribute to intracranial pressure elevation. However, there are equivocal results concerning the effect of angiotensin II on cerebral arteries, with both vasoconstriction (Edvinsson *et al.* 1979) and vasodilatation being described (Haberl *et al.* 1990). We found that I.C. administration of irbesartan failed to modify the level of intracranial pressure in rats not subjected to SAH. This observation is in agreement with clinical studies where converting enzyme inhibitors such as captopril (Schmidt *et al.* 1990) or ramipril (Demolis *et al.* 1992) exerted no effect on intracranial pressure. The exaggerated rise in intracranial pressure following SAH in I.C. irbesartan-treated animals is

surprising given the ability of angiotensin II to elevate intracranial pressure in rodents when injected centrally (Barbella *et al.* 1983; Morrow *et al.* 1992). While exogenous angiotensin II may have an effect on intracranial pressure in the intact brain, blockade of endogenous angiotensin II in SAH does not facilitate the anticipated restoration of intracranial pressure. On the contrary, angiotensin receptor blockade aggravates the elevation in intracranial pressure, thereby resulting in a reduction in cerebral perfusion pressure. Brain angiotensin II is believed to be of importance in the regulation of brain vascular permeability (Raichle, 1983) and disruption of the blood–brain barrier following SAH results in pronounced permeability changes (Johshita *et al.* 1990). It seems plausible that the increase in intracranial pressure we describe may reflect profound disturbances associated with the presence of blood in the subarachnoid space coupled with alterations in permeability controlled by the renin–angiotensin system.

Blockade of the renin–angiotensin system has proven to be beneficial in reversing cerebral vasospasm in experimental SAH (Andrews *et al.* 1982; Honda *et al.* 1997). Experiments in rats show that vasospasm develops as early as 5 min and is maintained for 3 days (Delgado *et al.* 1985). Most patients presenting vasospasm develop ischaemic symptoms associated with a decrease in cerebral perfusion. Indeed, the probability of an unfavourable neurological outcome is increased with decreasing cerebral perfusion pressure (Gruber *et al.* 1999). The present study demonstrates that the blockade of the AT_1 receptors, at least in the initial stage following SAH, would probably aggravate the state of the patients as it results in a combined unwanted drop in blood pressure and a rise in intracranial pressure. Rather than being at odds with studies indicating that blockade of the renin–angiotensin system prevents cerebral vasospasm (Andrews *et al.* 1982; Honda *et al.* 1997), our observation of a beneficial effect of angiotensin possibly reflects a different facet of the system's involvement in the pathophysiology associated with SAH. While we have demonstrated the importance of angiotensin II in the maintenance of cerebral perfusion pressure, whether such treatment, as advocated by our results, impacts on cerebral vasospasm in our model remains unknown.

Acikgoz, B., Ozgen, T., Ozdogan, F., Sungur, A. & Tekkok, I. H. (1996). Angiotensin II receptor content within the subfornical organ and organum vasculosum lamina terminalis increases after experimental subarachnoid haemorrhage in rats. *Acta Neurochirugica* **138**, 460–465.

Andrews, P., Papadakis, N. & Gavras, H. (1982). Reversal of experimental acute cerebral vasospasm by angiotensin converting enzyme inhibition. *Stroke* **13**, 480–483.

Astrup, J., Siesjo, B. K. & Symon, L. (1981). Thresholds in cerebral ischemia – the ischemic penumbra. *Stroke* **12**, 723–725.

Barbella, Y. R., Keil, L. C., Wurpel, J. N. & Severs, W. B. (1983). Cerebrospinal fluid pressure during cerebroventricular infusion of angiotensin and vasopressin. *Experimental Neurology* **82**, 325–334.

Bishop, V., Shade, R. & Haywood, J. (1991). Hormonal modulation of baroreceptor reflexes. In *Baroreceptor Reflexes*, ed. Persson, K. P., pp. 228–238. Springer-Verlag, New York.

Bishop, V. S. & Hay, M. (1993). Involvement of the area postrema in the regulation of sympathetic outflow to the cardiovascular system. *Frontiers in Neuroendocrinology* **14**, 57–75.

Brinker, T., Seifert, V. & Dietz, H. (1992). Cerebral blood flow and intracranial pressure during experimental subarachnoid haemorrhage. *Acta Neurochirugica* **115**, 47–52.

Brinker, T., Seifert, V. & Stolke, D. (1990). Acute changes in the dynamics of the cerebrospinal fluid system during experimental subarachnoid hemorrhage. *Neurosurgery* **27**, 369–372.

Broderick, J., Brott, T., Duldner, J., Tomsick, T. & Leach, A. (1994). Initial and recurrent bleeding are the major causes of death following subarachnoid hemorrhage. *Stroke* **25**, 1342–1347.

Culman, J., von Heyer, C., Piepenburg, B., Rascher, W. & Unger, T. (1999). Effects of systemic treatment with irbesartan and losartan on central responses to angiotensin II in conscious, normotensive rats. *European Journal of Pharmacology* **367**, 255–265.

Dampney, R. A. & Moon, E. A. (1980). Role of ventrolateral medulla in vasomotor response to cerebral ischemia. *American Journal of Physiology* **239**, H349–358.

Delgado, T. J., Brismar, J. & Svendgaard, N. A. (1985). Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* **16**, 595–602.

Demolis, P., Chalon, S., Annane, D., Duhaze, P. & Giudicelli, J. F. (1992). Effects of an angiotensin converting enzyme inhibitor, ramipril, on intracranial circulation in healthy volunteers. *British Journal of Clinical Pharmacology* **34**, 224–230.

Dorward, P. K. & Rudd, C. D. (1991). Influence of brain renin-angiotensin system on renal sympathetic and cardiac baroreflexes in conscious rabbits. *American Journal of Physiology* **260**, H770–778.

Edvinsson, L., Hardebo, J. E. & Owman, C. (1979). Effects of angiotensin II on cerebral blood vessels. *Acta Physiologica Scandinavica* **105**, 381–383.

Eisenhofer, G., Goldstein, D. S., Stull, R., Keiser, T., Sunderland, T., Murphy, D. L. & Kopin, I. (1986). Simultaneous liquid chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma and their responses to inhibition of monoamine oxidase. *Clinical Chemistry* **31**, 2030–2033.

Fassot, C., Lambert, G., Gaudet-Lambert, E., Friberg, P. & Elghozi, J. L. (1999). Beneficial effect of renin-angiotensin system for maintaining blood pressure control following subarachnoid haemorrhage. *Brain Research Bulletin* **50**, 127–132.

Fedina, L., Pasztor, E., Kocsis, B. & Berta, Z. (1986). Activity of peripheral sympathetic efferent nerves in experimental subarachnoid haemorrhage. Part II: Observations during the 'early vasospasm' period. *Acta Neurochirugica* **80**, 42–46.

Gaudet, E. A., Godwin, S. J. & Head, G. A. (1998). Role of central catecholaminergic pathways in the actions of endogenous ANG II on sympathetic reflexes. *American Journal of Physiology* **275**, R1174–1184.

Gebber, G. L. & Barman, S. M. (1985). Lateral tegmental field neurons of cat medulla: a potential source of basal sympathetic nerve discharge. *Journal of Neurophysiology* **54**, 1498–1512.

Grassi, G., Turri, C., Dell'Oro, R., Stella, M. L., Bolla, G. B. & Mancia, G. (1998). Effect of chronic angiotensin converting enzyme inhibition on sympathetic nerve traffic and baroreflex control of the circulation in essential hypertension. *Journal of Hypertension* **16**, 1789–1796.

Gruber, A., Reinprecht, A., Illievich, U. M., Fitzgerald, R., Dietrich, W., Czech, T. & Richling, B. (1999). Extracerebral organ dysfunction and neurologic outcome after aneurysmal subarachnoid hemorrhage. *Critical Care Medicine* **27**, 505–514.

Haberl, R. L., Anneser, F., Villringer, A. & Einhaupl, K. M. (1990). Angiotensin II induces endothelium-dependent vasodilation of rat cerebral arterioles. *American Journal of Physiology* **258**, H1840–1846.

Honda, Y., Minato, H., Fujitani, B., Masuda, Y. & Hosoki, K. (1997). Alacepril, an angiotensin-converting enzyme inhibitor, prevents cerebral vasospasm in subarachnoid hemorrhage model in rats. *Methods and Findings in Experimental and Clinical Pharmacology* **19**, 699–706.

Johshita, H., Kassell, N. F. & Sasaki, T. (1990). Blood-brain barrier disturbance following subarachnoid hemorrhage in rabbits. *Stroke* **21**, 1051–1058.

Lambert, E., Lambert, G., Fassot, C., Friberg, P. & Elghozi, J. L. (2000). Subarachnoid hemorrhage induced sympathoexcitation arises due to changes in endothelin and/or nitric oxide activity. *Cardiovascular Research* **45**, 1046–1053.

Lambert, G., Lambert, E., Fassot, C., Friberg, P. & Elghozi, J. L. (2001). Subarachnoid haemorrhage-induced sympathoexcitation in rats is reversed by bosentan or sodium nitroprusside. *Clinical and Experimental Pharmacology and Physiology* **28**, 200–205.

Martineau, D., Lamouche, S., Briand, R. & Yamaguchi, N. (1999). Functional involvement of angiotensin AT2 receptor in adrenal catecholamine secretion in vivo. *Canadian Journal of Physiology and Pharmacology* **77**, 367–374.

Moreau, N., Richer, C., Vincent, M. P. & Giudicelli, J. F. (1993). Interaction between SR 47436, a new angiotensin II antagonist and sympathetic nervous system in pithed SHR rats. *Archives des Maladies du Coeur, des Vaisseaux et du sang* **86**, 1269–1274.

Morrow, B. A., Keil, L. C. & Severs, W. B. (1992). Resistance to outflow of cerebrospinal fluid after central infusions of angiotensin. *Proceedings of the Society for Experimental Biology and Medicine* **199**, 34–37.

Naredi, S., Lambert, G., Eden, E., Zall, S., Runnerstam, M., Rydenhag, B. & Friberg, P. (2000). Increased sympathetic nervous activity in patients with nontraumatic subarachnoid hemorrhage. *Stroke* **31**, 901–906.

Neil-Dwyer, G., Walter, P., Shaw, H. J., Doshi, R. & Hodge, M. (1980). Plasma renin activity in patients after a subarachnoid hemorrhage – a possible predictor of outcome. *Neurosurgery* **7**, 578–582.

Peach, M. J. (1977). Renin-angiotensin system: biochemistry and mechanisms of action. *Physiological Reviews* **57**, 313–370.

Peach, M. J., Bumpus, F. M. & Khairallah, P. A. (1969). Inhibition of norepinephrine uptake in hearts by angiotensin II and analogs. *Journal of Pharmacology and Experimental Therapeutics* **167**, 291–299.

Proescholdt, M. G., Hutto, B., Brady, L. S. & Herkenham, M. (2000). Studies of cerebrospinal fluid flow and penetration into brain following lateral ventricle and cisterna magna injections of the tracer [^{14}C]inulin in rat. *Neuroscience* **95**, 577–592.

Raichle, M. E. (1983). Neurogenic control of blood-brain barrier permeability. *Acta Neuropathology Supplement* **8**, 75–79.

Rasmussen, G., Hauerberg, J., Waldemar, G., Gjerris, F. & Juhler, M. (1992). Cerebral blood flow autoregulation in experimental subarachnoid haemorrhage in rat. *Acta Neurochirugica* (*Wien*) **119**, 128–133.

Reid, I. A. (1992). Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *American Journal of Physiology* **262**, E763–778.

Rongen, G. A., Brooks, S. C., Ando, S., Abramson, B. L. & Floras, J. S. (1998). Angiotensin AT1 receptor blockade abolishes the reflex sympatho-excitatory response to adenosine. *Journal of Clinical Investigation* **101**, 769–776.

Schmidt, J. F., Andersen, A. R., Paulson, O. B. & Gjerris, F. (1990). Angiotensin converting enzyme inhibition, CBF autoregulation, and ICP in patients with normal-pressure hydrocephalus. *Acta Neurochirugica* **106**, 9–12.

Schmitt, F., Martinez, F., Brillet, G., Nguyen-Khoa, T., Brouard, R., Sissmann, J., Lacour, B. & Grunfeld, J. P. (1998). Acute renal effects of AT1-receptor blockade after exogenous angiotensin II infusion in healthy subjects. *Journal of Cardiovascular Pharmacology* **31**, 314–321.

Sun, M. K. & Reis, D. J. (1994). Hypoxia selectively excites vasomotor neurons of rostral ventrolateral medulla in rats. *American Journal of Physiology* **266**, R245–256.

Tsutsumi, K. & Saavedra, J. M. (1991). Quantitative autoradiography reveals different angiotensin II receptor subtypes in selected rat brain nuclei. *Journal of Neurochemistry* **56**, 348–351.

van Wylen, D. G. & D'Alecy, L. G. (1985). Regional blood flow distribution during the Cushing response: alterations with adrenergic blockade. *American Journal of Physiology* **248**, H98–108.

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